REMARKS

Applicants respectfully request consideration of the application in view of the foregoing amendments and the following remarks.

Claims 1, 11, 20, and 22 are amended herein to more particularly point out and distinctly claim the subject matter of Applicants' invention. Support for the amendments can be found, *inter alia*, in original claims 8, 10, and 12. Claims 4-5, 7, and 17-18 are amended herein to clarify that the term "host cell," as claimed, does not encompass a cell within a multi-cellular organism. Claims 4, 9, 15, and 16 are amended herein to remove the previous dependency on a now canceled claim. No new matter has been added.

Claims 8, 10, and 12 are canceled herein without prejudice to pursuing the subject matter of said claims in a later filed divisional application(s).

Claim Objections

Claim 4 is objected to as depending from a cancelled claim. In response thereto, Applicants have amended claim 4 herein to change the dependency to pending claim 2. Accordingly, Applicants respectfully request that the objection to claim 4 be removed and the claim allowed.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-2, 4-5, 7-18, 20, 22 and 24 are rejected under 35 U.S.C. § 112, paragraph one, as failing to comply with the written description requirement. It is alleged that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time the invention was filed. Applicants respectfully traverse.

Specifically, the Office Action states that independent claims 1, 11, 20, and 22 are directed to synthetic nucleic acid molecules encoding any mammalian heparanase protein. It is alleged that the specification teaches the structure of only a few representative species of such synthetic nucleic acid molecules of SEQ ID NO:19 encoding human heparanase protein SEQ ID NO:16. It is further alleged that the specification fails to describe any other species by any identifying characteristics other than the functionality of encoding mammalian heparanase.

Applicants note that the cited claims are amended herein to limit the claimed sequences to human heparanase sequences, which have been engineered in a specific manner to allow expression of

biologically active human heparanase in non-mammalian cells. The nucleotide and amino acid sequences of wild-type human heparanase are known in the art, as described in the specification on page 6, lines 11-21 (citing Vlodavksy et al, *Nature Med.* 5: 793-802 (1999); Hulett et al, *Nature Med.* 5: 803-809 (1999); Toyoshima & Nakajima, *J. Biol. Chem.* 274(34): 24153-24160 (1999); and Genbank Accession No. AF155510). The active form of heparanase was previously proposed to be a heterodimer between a 50 kDa C-terminal fragment and an 8 kDa N-terminal fragment, arising from the excision of an intervening 6 kDa peptide by unidentified proteolytic enzyme(s). The human heparanase sequence, as described in claim 1 and method claim 20, was modified by Applicants to contain consensus cleavage sites recognized by a specific known protease, which allows the excision of a 6 kDa "intervening fragment" by the known protease, leading to biologically active heparanase. The human heparanase sequence, as described in claim 11 and method claim 22, was modified by Applicants to encode the 8 kDa N-terminal fragment and the C-terminal 50 kDa fragment, separated by a heterologous linker. Said nucleic acid molecule encodes a form of human heparanase that is constitutively active.

Therefore, the cited claims are limited to human heparanase sequences which contain specific modifications that allow expression of biologically active enzyme in heterologous expression systems. As such, Applicants respectfully submit that the rejection of claims 1-2, 4-5, 7-18, 20, 22 and 24, under §112, first paragraph, as failing to comply with the written description requirement, is overcome. Accordingly, Applicants respectfully request that the rejection be removed and the claims allowed.

Claims 1-2, 4-5, 7-18, 20, 22 and 24 are rejected under 35 U.S.C. § 112, paragraph one, because the specification allegedly does not enable any person skilled in the art to which it pertains, to make and use the invention commensurate in scope with the claims. Specifically, the Office Action alleges that the specification "while being enabling for a synthetic nucleic acid molecule of SEQ ID NO:19 comprising a sequence of nucleotides that encodes a heparanase protein of SEQ ID NO:16, does not reasonably provide enablement for a synthetic nucleic acid molecule comprising a sequence of nucleotides that encodes any mammalian heparanase protein from any source." *See* Office Action at page 4, paragraph 2. Applicants respectfully traverse.

The Supreme Court has interpreted the enablement requirement set forth in the first paragraph of § 112 to require that, at the time of filing, one of skill in the art is able to practice the claimed invention without undue or unreasonable experimentation. *Mineral Separation v. Hyde* 242 U.S. 261, 270 (1916). Applicants respectfully submit that the full scope of the present claims is enabled by

Applicants specification because one of skill in the art would be able to practice the claimed invention without undue experimentation.

First, Applicants note that the claims, as amended, are limited to human heparanase sequences with specific engineered modifications, as discussed above. At the time of filing the present application, the wild-type nucleotide and amino acid sequences of human heparanase were known and available in the art (Vlodavksy et al, *supra*; Hulett et al, *supra*; Toyoshima & Nakajima, *supra*; and Genbank Accession No. AF155510). Further, Applicants' specification provides ample guidance to one of skill in the art to modify these wild-type sequences in accordance with the claimed invention, as described below.

The determination of wild-type heparanase sequences allowed the recombinant expression of human heparanase; however, before the instant invention, it was not possible to express high levels of stable, correctly processed, recombinant human heparanase in non-mammalian cells. Although it was postulated in the art that the active form of heparanase consists of a heterodimer between an N-terminal 8kDa fragment and a C-terminal 50 kDa fragment of heparanase, it was unknown what proteolytic enzyme was responsible for excising the 6 kDa intervening fragment. Attempts to express recombinant human heparanase in heterologous non-mammalian expression systems led to protein expression, but the resulting protein was not biologically active. Active heparanase could be obtained by purifying the processed protein from human cells, such as platelets (*see, e.g.* Fairbanks *et al.* J. *Biol. Chem.* 274:24153-24160(1999) and U.S. Patent 6,387,643, columns 15-16), but yields of endogenous heparanase were very low.

In accordance with the present invention, Applicants solved the problems of expressing high-levels of biologically active recombinant heparanase in heterologous non-mammalian expression systems in two different ways. First, as described in independent claims 1 and 20, Applicants engineered nucleic acid molecules encoding human heparanase to contain two consensus cleavage sites recognized by a *known* protease between nucleotides encoding residues 100 and 168 of human heparanase. Such a nucleic acid molecule can be used to efficiently express heparanase in non-mammalian heterologous cells, which can then be cleaved by the known protease to substantially remove the 6 kDa "intervening fragment," resulting in biologically active heparanase. The second means by which Applicants solved the problems described above, was by providing a nucleic acid molecule encoding the N-terminal 8kDa fragment of heparanase and the C-terminal 50 kDa fragment of heparanase, connected by a heterologous linker. Such a construct encodes a human heparanase protein that is constitutively active in heterologous cells, resulting in high yields of biologically active protein.

Applicants' specification describes the production of synthetic nucleic acid molecules that encode human heparanase protein having two heterologous consensus cleavage sites recognized by a known, defined endoproteinase selected from a list, such that the known endoproteinase can be used to substantially excise the 6 kDa "intervening fragment," resulting in biologically active human heparanase. In that regard, the Specification describes the introduction of protease cleavage sites (see, e.g. Specification at page 10, line 24- page 11, line 3) and the construction and subsequent expression of such nucleic acid molecules (*see, e.g.*, Examples 1 and 3-7). Applicants' specification also describes the production and expression of nucleic acid molecules encoding the N-terminal 8kDa fragment of heparanase and the C-terminal 50 kDa fragment of heparanase, connected by a heterologous linker, such nucleic acid molecules encoding modified human heparanase proteins that are constitutively active. Specific guidance in that regard can be found, *inter alia*, on page 11, lines 4-21, and in Examples 2-7. Using the current specification as a guide, in conjunction with human heparanase sequences well known in the art, production of a synthetic nucleic acid molecule in accordance with the invention could be easily accomplished by one of skill in the art.

For the reasons set forth above, Applicants respectfully assert that it would not require undue experimentation to practice the claimed invention. Accordingly, Applicants assert that the above-cited claims are in condition for allowance and respectfully request that the rejection of these claims under the enablement requirement of §112, paragraph 1, be removed and the claims allowed.

Claims 4-5, 7, and 17-18, are rejected under 35 U.S.C. § 112, paragraph one, because the specification allegedly does not enable any person skilled in the art to which it pertains, to make and use the invention commensurate in scope with the claims. Specifically, the Office Action alleges that the specification "while being enabling for isolated host cells transformed with the recited nucleic acids does not reasonably provide enablement for host cells within a multicellular animal." *See* Office Action at page 5, paragraph 2. Applicants respectfully traverse.

Applicants respectfully assert that the cited claims are enabled by Applicants' specification. Claims 4-5, 7, and 17-18 are amended herein to add the term "isolated" before the term "host cell" to clarify that the claims do not encompass a cell within a multi-cellular organism. Accordingly, Applicants respectfully request that the rejection of the cited claims under the enablement requirement of §112, paragraph 1, be removed and the claims allowed.

Rejections under 35 U.S.C. § 102

Claims 1-2, 4-5, 7-18, 20, 22 and 24 are rejected under 35 U.S.C. § 102(b), as being anticipated by Heinrikson *et al.* (U.S. Patent No. 6,387,643, hereinafter "Heinrikson"). The disclosure of Heinrikson is alleged to teach a fusion protein comprising human heparanase, which encodes pro-heparanase protein having two cleavage sites for proteolytic cleaving propeptide to active processed heparanase protein, which is located at amino acid glu96-ser97 and glu144-lys145 of human heparanase protein. Applicants respectfully traverse.

In order to anticipate a claim, a single prior art source must contain all of the essential limitations of the claim. *See, e.g. Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 USPQ 81 (Fed. Cir. 1986) ("for prior art to anticipate under § 102 it has to meet every element of the claimed invention"). Applicants respectfully submit that the cited claims are not anticipated by Heinrikson because each of the claim limitations of independent claims 1, 11, 20, and 22 are not taught by Heinrikson.

Applicants note that the cited claims require nucleic acid molecules encoding *specific, modified* forms of human heparanase. Heinrikson discloses the nucleotide sequence of unmodified, human heparanase. While the wild-type heparanase sequence disclosed in Heinrikson comprises consensus cleavage sites recognized by an endoproteinase, it is unknown which endoproteinase recognizes the cleavage sites of the wild-type protein. Heinrikson fails to teach a nucleic acid molecule comprising a sequence of nucleotides that encodes a human heparanase protein that is engineered to contain two consensus cleavage sites recognized by a specific, known protease, wherein the cleavage sites are selected from the group consisting of: tobacco etch virus (TEV) protease cleavage sites, 3C protease cleavage sites from picornavirus, thrombin protease cleavage sites, enterokinase cleavage sites and factor Xa cleavage sites, as required by claims 1 and 20.

Heinrikson also fails to teach nucleic acid molecules encoding the N-terminal 8kDa fragment of heparanase and the C-terminal 50 kDa fragment of heparanase, connected by a heterologous linker, as required by claims 11 and 22. Applicants respectfully note that the term "linker" is defined in Applicants specification as being either "*synthetic* or *isolated* from a naturally occurring source" (see Specification at page 11, lines 17-18, emphasis added). This definition would exclude the sequence encoding the 6kDa fragment of heparanase that is originally present in wild-type proheparanase, which is not synthetic nor isolated. Moreover, claims 11 and 22 require that the claimed nucleic acid molecules encode a human heparanase that is constitutively active. The wild-type pro-heparanase nucleotide sequence that encodes the 8 and 50 kDa fragments of human heparanase joined by the natural 6kDa intervening fragment is not active until further processing occurs.

Applicants respectfully disagree with the assertion in the Office Action that Heinrikson teaches the cleaving of purified human heparanase by thrombin. There are two disclosures of the enzyme "thrombin" in Heinrikson, but neither disclosure teaches or suggests the cleaving of purified human heparanase by thrombin. "Thrombin" first appears in Heinrikson in column 15, lines 1-6, where it is stated that human platelets were "stimulated with 1 U/ml thrombin for 5 min at 37° C." It is further stated that "[t]his concentration of thrombin has been reported to release 100% of the heparanase activity from platelets." This disclosure of thrombin clearly describes the use of thrombin to release heparanase from platelets at the cell surface. Use of thrombin, in this instance, would not lead to processing of heparanase because thrombin cleavage sites are not present at the boundary of the 6 kDa "intervening fragment" in naturally occurring heparanase.

Similarly, in column 23, Heinrikson describes the cloning of a PCR product into a cloning vector which contains "[a] protein kinase site for phosphorylation and a thrombin site for excision of the recombinant protein..." (see column 23, lines 34-36). This disclosure describes a common feature of cloning vehicles that allow use of thrombin to release protein from a linker or leader. It does not describe the intramolecular processing of heparanase with thrombin or the addition of thrombin cleavage sites at or near the boundary of the intervening fragment that is excised from active, mature heparanase.

Therefore, Applicants respectfully submit that each of the claim limitations of independent claims 1, 11, 20, and 22 are not taught by Heinrikson. Accordingly, Applicants respectfully request that the rejection of the claims 1-2, 4-5, 7-18, 20, 22 and 24 under 35 U.S.C. §102(b) be removed and the claims allowed.

Claims 1-2, 4-5, 7-18, 20, 22 and 24 are further rejected under 35 U.S.C. § 102(b), as being anticipated by Levy-Adam *et al.* (*Biochem. Biophys. Res. Commun.* 308(4): 885-91 (2003), hereinafter "Levy-Adam"). The disclosure of Levy-Adam is alleged to teach a fusion protein comprising human heparanase, which encodes pro-heparanase protein having two cleavage sites for proteolytic cleaving propeptide to active processed heparanase protein, which is located at amino acid glu109-ser110 and glu157-lys158 of human heparanase protein with a linking peptide between two fragments of 8 kDa and 50 kDa. Applicants respectfully traverse.

As noted above, in order to anticipate a claim, a single prior art source must contain all of the essential limitations of the claim. *See, e.g. Hybritech, Inc., supra.* Applicants respectfully submit that the cited claims are not anticipated by Levy-Adam because each of the claim limitations of independent claims 1, 11, 20, and 22 are not taught therein.

Applicants reiterate the analysis set forth above with respect to Heinrikson, which also describes the deficiencies of the disclosure of Levy-Adam in anticipating the claimed invention. To summarize, Levy-Adam fails to teach a nucleic acid molecule comprising a sequence of nucleotides that encodes a human heparanase protein that is engineered to contain two consensus specifically defined cleavage sites recognized by a known protease, as required by claims 1 and 20. Levy-Adam also fails to teach nucleic acid molecules encoding the N-terminal 8kDa fragment of heparanase and the C-terminal 50 kDa fragment of heparanase, connected by a heterologous linker, and encoding a constitutively active protein, as required by claims 11 and 22.

Applicants respectfully disagree with the assertion in the Office Action that Levy-Adam teaches the cleaving of purified human heparanase by thrombin. There is a single disclosure of the enzyme "thrombin" in Levy-Adam, which states:

> HSPGs tether growth factors, cytokines, and chemokines to the ECM as high affinity storage depots for bioactive molecules [ ]. The release of such potent regulators is likely to alter the functional state of cells and tissues and, therefore, might be tightly regulated. *HSPG shedding* can be catalyzed by proteases, such as thrombin or plasmin, which recognize basic cleavage sites in the HSPG core protein, or by an endoglycosidase, heparanase, which specifically cleaves HS side chains.

See Levy-Adam, page 890, paragraph 1, emphasis added.

The discussion of thrombin in Levy-Adam clearly describes the release of heparin sulfate proteoglycans (HSPGs) from biological regulators in the extracellular matrix. It does not describe the use of thrombin for the intramolecular processing of heparanase, allowing the conversion of inactive proheparanase to the active form of the enzyme. Levy-Adam also does not teach the addition of thrombin cleavage sites within the heparanase molecule so that thrombin could be used to stimulate enzymatic activity. In fact, Levy-Adam make it clear the "the protease(s) responsible for converting the ~65 kDa inactive heparanase precursor into its 50 kDa (heterodimer) active form have not been identified."

Therefore, Applicants respectfully submit that each of the claim limitations of independent claims 1, 11, 20, and 22, are not taught by Levy-Adam. Accordingly, Applicants respectfully request that the rejection of claims 1-2, 4-5, 7-18, 20, 22 and 24 under 35 U.S.C. §102(b) be removed and the claims allowed.

Summary

Applicants assert all claims are in condition for allowance and a favorable action on the merits is earnestly solicited.

If the Examiner believes that a telephone conference would be of value, he is requested to call the undersigned attorney at the number listed below.

Respectfully submitted,

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